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L4: Entry 6 of 138 File: PGPB Dec 9, 2004

DOCUMENT-IDENTIFIER: US 20040248784 A1
TITLE: Unitary combinations of FSH and hCG

Detail Description Paragraph:
[0042] It can be advantageous to employ FSH isoforms that differ in the extent to which they are post-translationally modified. Due to different modifications, the isoforms exhibit differences in overall charge, degree of sialic acid (a terminal sugar) or sulfate incorporation, receptor binding affinity and plasma half-life. Chappel et al., Endocrine Reviews 4:179 (1983); Snyder et al. Mol. Cell. Endocrin. 54:115 (1987). These forms are separable from each other on the basis of their overall charge and all isoforms exhibit biological activity. Isoforms that exhibit a greater net negative charge are more heavily sialylated, exhibit a longer metabolic clearance rate and a greater biologic activity due to their extended plasma survival time.

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L3: Entry 8 of 25 File: PGPB Jul 3, 2003

DOCUMENT-IDENTIFIER: US 20030124737 A1
TITLE: Diagnostic kit for predicting pregnancy outcome

CLAIMS:

1. A method of predicting pregnancy outcome in a subject by determining the amount of an early pregnancy associated molecular isoform of hCG in a sample comprising: (a) contacting a sample with an antibody which specifically binds to the early pregnancy associated molecular isoform of hCG under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG; (b) measuring the amount of complexes formed, thereby determining the amount of the early pregnancy associated molecular isoform of hCG in the sample; and (c) comparing the amount early pregnancy associated molecular isoform of hCG in the sample determined in step (b) with either (i) the amount determined for temporally matched, normal pregnant subject(s) or (ii) the amount determined for non-pregnant subject(s), wherein the relative absence of the early pregnancy associated molecular isoform of hCG in the sample indicates a negative outcome of pregnancy for the subject.

3. The method of claim 1, step (a) further comprising a second antibody which specifically binds to hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG.

5. The method of claim 1, step (a) further comprising a second antibody which specifically binds to intact non-nicked hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG.

7. The method of claim 5, step (c) comprising comparing the amount of the early pregnancy associated molecular isoform of hCG determined in step (b) for said antibody with the amount determined in step (b) for the second antibody, wherein a high ratio of amounts determined for said antibody relative to the second antibody indicates a positive outcome of pregnancy for the subject, a low ratio indicates a negative outcome of pregnancy for the subject.

8. The method of claim 1, step (c) comprising comparing the amount early pregnancy associated molecular isoform of hCG in the sample determined in step (b) with either either (i) the amount determined for temporally matched, normal pregnant subject(s) or (ii) the amount determined for non-pregnant subject(s), wherein amounts of the early pregnancy associated molecular isoform of hCG in the sample similar to amounts amounts of early pregnancy associated molecular isoform of hCG in temporally matched pregnant samples indicates a positive outcome, amounts of early pregnancy associated molecular isoform of hCG in the sample similar to amounts of early pregnancy associated molecular isoform of hCG in the non-pregnant samples indicates a negative outcome of pregnancy for the subject.

14. A method of predicting pregnancy outcome in a subject by determining the amount of an early pregnancy associated molecular isoform of hCG in a sample comprising:

(a) contacting a capturing antibody which specifically binds to the early pregnancy associated molecular isoform of hCG with a solid matrix under conditions permitting binding of the antibody with the solid matrix; (b) contacting the bound matrix with the sample under conditions permitting binding of the antigen present in the sample with the capturing antibody; (c) separating the bound matrix and the sample; (d) contacting the separated bound matrix with a detecting antibody which specifically binds to hCG under conditions permitting binding of antibody and antigen in the sample; (e) measuring the amount of bound antibody on the bound matrix, thereby determining the amount of early pregnancy associated molecular isoform of hCG in the sample; (f) comparing the amount early pregnancy associated molecular isoform of hCG in the sample determined in step (e) with either (i) the amount determined for temporally matched, normal pregnant subject(s) or (ii) the amount determined for non-pregnant subject(s), wherein amounts of the early pregnancy associated molecular isoform of hCG in the sample similar to amounts of early pregnancy associated molecular isoform of hCG in temporally matched pregnant samples indicates indicates a positive outcome, amounts of early pregnancy associated molecular isoform of hCG in the sample similar to amounts of early pregnancy associated molecular isoform of hCG in the non-pregnant samples indicates a negative outcome of of pregnancy for the subject.

18. The method of claim 14, step (a) further comprising a second capturing antibody which specifically binds to intact non-nicked hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG.

20. The method of claim 14, step (d) further comprising a second detecting antibody which specifically binds to hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG.

21. The method of claim 14, step (f) comprising comparing the amount of the early pregnancy associated molecular isoform of hCG determined in step (e) for said antibody with the amount determined in step (b) for the second antibody, wherein a high ratio of amounts determined for said antibody relative to the second antibody indicates a positive outcome of pregnancy for the subject, a low ratio indicates a negative outcome of pregnancy for the subject.

27. A method for determining the amount of early pregnancy associated molecular isoforms of in a sample comprising: (a) contacting the sample with an antibody which which specifically binds to an early pregnancy associated molecular isoform of hCG under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG; and (b) determining the amount of complexes formed thereby determining the amount of early pregnancy associated-molecular isoform of hCG in the sample.

28. The method of claim 27, wherein the antibody specifically binds a region of the early pregnancy associated molecular isoform of hCG comprising a carbohydrate moiety.

31. A diagnostic kit for determining the amount of early pregnancy associated hCG is a sample comprising: (a) An antibody which specifically binds to an early pregnancy associated molecular isoform; and (b) a solid matrix to which the antibody antibody is bound; and (c) reagents permitting the formation of a complex between the antibody and a sample.

37. An antibody which specifically binds to an early pregnancy associated molecular isoform of human chorionic gonadotropin.

38. The antibody of claim 37, wherein the antibody specifically binds to a region of the early pregnancy associated molecular isoform of human chorionic gonadotropin

comprising a carbohydrate moiety.

41. The early pregnancy associated isoform of hCG of claim 1.

42. The early pregnancy associated isoform of hCG recognized by the monoclonal antibody of claim 39.

43. A method for detecting non-trophoblast malignancy in a sample comprising: (a) contacting a sample with an antibody which specifically binds to the early pregnancy associated molecular isoform of hCG under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG; (b) contacting the sample with a second antibody which specifically binds to intact non-nicked hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG. (c) measuring the amount of complexes formed, thereby determining the amount of the early pregnancy associated molecular isoform of hCG in the sample; and (d) comparing the amount of early pregnancy associated molecular isoform of hCG in the sample determined in step step (b) with the amount of early pregnancy associated molecular isoform of hCG in the sample determined in step (c), wherein a positive detection of early pregnancy associated molecular isoform detected in step (b) and a relative absence of the early pregnancy associated molecular isoform of hCG detected in step (c) indicates the presence of non-trophoblast malignancy in the sample.

48. A method for detecting gestational trophoblast disease in a sample from a subject comprising: (a) contacting a sample with an antibody which specifically binds to the early pregnancy associated molecular isoform of hCG under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG; (b) contacting the sample with a second antibody which specifically binds to intact non-nicked hCG without substantially cross-reacting with said antibody under conditions permitting formation of a complex between the antibody and the early pregnancy associated molecular isoform of hCG; (c) measuring the amount of complexes formed, thereby determining the amount amount of the early pregnancy associated molecular isoform of hCG in the sample due to binding with the first antibody, and late pregnancy associated molecular isoform of hCG in the sample due to binding with the second antibody; (d) determining the ratio of early pregnancy associated molecular isoform of hCG to late pregnancy associated molecular isoform of hCG in the subject; and (e) comparing the ratio of early pregnancy associated molecular isoform of hCG to late pregnancy associated molecular isoform of hCG in the sample determined in step (c) over time, wherein a continuing high ratio of early pregnancy associated molecular isoform of hCG to late pregnancy associated molecular isoform of hCG in the sample determined in step (c) indicates the presence of gestational trophoblast disease in the subject.

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L3: Entry 15 of 25 File: PGPB Apr 11, 2002

DOCUMENT-IDENTIFIER: US 20020042927 A1
TITLE: Meiosis activating sterol augments implantation rate

CLAIMS:

7. Use according to claims 1 or 2, wherein the additive or additives leads to a ratio of at least 2 between the relative content of MAS in cumulus enclosed oocytes cultured in the presence of the additive or additives, the relative content of MAS in cultured cumulus enclosed oocytes being determined by stimulation female mice with exogenous gonadotropins 48h prior to removal of the ovaries from the mice and recovering cumulus enclosed oocytes from the ovaries by puncturing individual follicles and culturing the recovered cumulus enclosed oocytes in an .alpha.-MEM medium supplemented with 3mg/l bovine serum albumin, 5 mg/l human serum albumin, 2mM L-glutamin, 100 IU/mI penicillin, 100.mu.g/ml steptomycin, 4mM hypoxanthine and .sup.3H-mevalonat for 24h at 37.degree. C., 100% humidity and 5% CO.sub.2 in air, followed acidification with 50.mu.l 0.3M Na.sub.2PO.sub.4 pH=1, organic extraction three times with a five-fold surplus of n-heptane:isopropanol (3:1 v/v), purification of MAS from the organic phase by HPLC and determination of the ratio of radioactivity per cumulus enclosed oocyte between cumulus enclosed oocytes cultured in the presence of the additive or additives and cumulus enclosed oocytes cultured without the presence of the additive or additives.

8. Use according to claim 7, wherein the additive is selected from the group consisting of gonadotropins such as FSH and analogues, growth hormones such as EGF and analogues, compounds inhibiting sterol .DELTA.14-reductase such as AY9944-A-7, compounds inhibiting 4-demethylase converting T-MAS to Zymosterol, compounds activating cytochrome P450 lanosterol 14.alpha.-demethylase and compounds with an amphotericin like effect.

9. Use according to claim 8, wherein the additive is a combination of a gonadotropin and a growth hormone.

10. Use according to claim 9, wherein the additive is a combination of EGF and FSH.

12. Use according to claim 8, wherein the additive is FSH.

13. Use according to claim 10 or 12, wherein FSH is an FSH isoform with an isoelectric point above 5.0.

14. Use according to any of claims 10, 12 or 13 wherein the FSH is derived from naturally occurring FSH such as FSH extracted from urine, or from recombinant FSH.

15. Use according to any of claims 10 or 12-14, wherein the concentration of FSH is between 2 and 200 IU FSH/I.

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L3: Entry 25 of 25 File: USPT Feb 11, 1992

DOCUMENT-IDENTIFIER: US 5087615 A
TITLE: Novel method of ovulation induction in humans

CLAIMS:

1. In a method for stimulating follicle development and ovulation in a female patient by administering FSH to said patient during the follicular phase of the ovulatory cycle, the improvement comprising initially administering a first FSH isoform having a relatively long plasma half-life and subsequently administering a second FSH isoform having a shorter plasma half-life.

2. The method of claim 1 wherein said first FSH isoform has an isoelectric focusing point less than about 4.3.

3. The method of claim 1 wherein said first FSH isoform is administered during the first three to five days of the ovulatory cycle.

4. The method of claim 1 comprising administering an intermediate FSH isoform having an intermediate plasma half-life after the administration of the first FSH isoform and prior to the administration of the second FSH isoform.

5. The method of claim 4 wherein said first FSH isoform has an isoelectric focusing point less than about 4.3, said intermediate FSH isoform has an isoelectric focusing point between about 4.3 and 5.4, and said second FSH isoform has an isoelectric focusing point greater than about 5.5.

6. The method of claim 4 wherein said first FSH isoform is administered during the first three to five days of the ovulatory cycle.

7. The method of claim 6 wherein the intermediate FSH isoform is administered during days six to 10 of the ovulatory cycle.

8. The method of claim 7 wherein the second FSH isoform is administered during the final days of the follicular phase of the ovulatory cycle.

9. In a method for stimulating follicle development and ovulation in a female patient by administering FSH to said patient during the follicular phase of the ovulatory cycle, the improvement comprising initially administering an FSH isoform characterized by relatively long plasma half-life, more sialylation and more acidic isoelectric focusing point followed by administering in one or more step increments FSH isoforms of progressively shorter plasma half-life, lesser sialylation, and more more alkaline isoelectric focusing point.

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